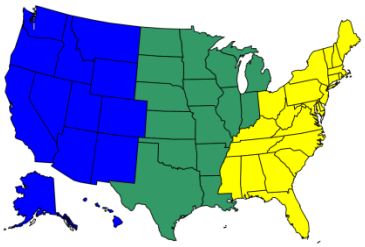
National Wildlife Health Center
Guidelines for Post-Emergence
Bat Submission
Summer/Fall 2010 (June-October)



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Purpose: The primary mission of the National Wildlife Health Center (NWHC) is to determine the cause of death in free-ranging, migratory wildlife involved in unusual die-off events. Since the emergence of White-nose Syndrome (WNS) among hibernating cave bats in the northeastern US, our lab has targeted sample collection to develop diagnostic tests to more rapidly and accurately identify affected sites. Because characteristic clinical signs observed in the winter are not readily apparent in summer bats, surveillance/early detection of WNS in new states is challenging. It requires large number of samples for which there are few labs able to perform the current diagnostics for WNS and it is not yet known if these assays can detect evidence of WNS in bats collected during the summer. Therefore, NWHC is focusing efforts to: 1) investigate unusual morbidity and mortality events in bats throughout the US, 2) assist with planned targeted surveillance for suspect bats in new locations, and 3) evaluate new bat species with wing damage in WNS-confirmed areas in conjunction with planned population monitoring activities. Limited evaluation of suspect bats from other areas will be considered based on the guidelines below. It should be stressed that only bats fitting the following criteria that are observed during planned surveillance or monitoring activities should be considered for diagnostic submission to our laboratory. As more observational data becomes available, summer and fall submission criteria may be revised.

Be sure to comply with all Federal and State permits (or authorizations) when capturing and handling bats. These guidelines do not supersede permit requirements.

Unusual mortality of bats observed in any State or US Territory

1. Dead bats found in greater numbers than normally expected for the species, age class, location, or time of year are being accepted at the NWHC for diagnostic evaluation with prior approval. This includes investigating increased adult and/or pup mortalities at maternity colonies throughout the US.

2. Collect 3-5 of the freshest carcasses (intact body, no evidence of scavenging, fur does not pull out easily, wings remain pliable, skin of face not dry or desiccated) which are representative of the affected species at a given site. Follow carcass collection and shipping instructions described at: http://www.nwhc.usgs.gov/mortality_events/reporting.jsp. Keep individual carcasses chilled in separate labeled bags indicating:
    - date died & date collected (if different)
    - location name(nearest town, county, state)
    - collector name & phone number
    - species
    - unique animal ID number (standard format: state, MMDDYY, collector, ###; ex: WI061610AEB001)
    - found dead or method of euthanasia

Group all individually bagged carcasses destined for laboratory shipment into a second clean bag prior to placing samples into a field cooler or traveling to additional sites.

3. If unable to ship chilled specimens within 48 hours of death for delivery to the lab no later than Thursday, freeze the carcasses and ship early the following week. ***NOTE:*** *The general public should be discouraged from handing any live bats due to the risk of rabies exposure and should be instructed to not directly contact any dead bats that they may wish to have examined. If willing, instruct public to use a disposable glove or a bag placed over their hand as a protective covering to pick up the carcass if they are certain that the bat is dead. The bat carcasses should then be double bagged in re-sealable plastic bags and packed on ice inside a cooler (not a food freezer) as soon as possible until you can arrive. Styrofoam coolers cannot be thoroughly disinfected and should be discarded after specimen retrieval while plastic coolers may be cleaned and decontaminated by following the guidelines described in* "Disinfection Protocol for Bat Field Research/Monitoring, June 2009"; http://www.fws.gov/WhiteNoseSyndrome/research.html, Protocols

4. Contact USGS-NWHC to arrange shipment and further instruction. For Eastern states, contact Dr. Anne Ballmann (608-270-2445; *aballmann@usgs.gov*). *Central states should contact Dr. LeAnn White, (608-270-2491; clwhite@usgs.gov). Western states should contact Krysten Schuler (608-270-2447; kschuler@usgs.gov).*

Any bat species with suspicious fungal growth on muzzle, ears, or wing membranes captured between June – October in any state

It is not anticipated to observe Geomyces destructans growth on bats during the summer or fall, however, we do not rule out the possibility. Please ensure any suspicious substance has a fuzzy, fungal-like appearance and is not dust, mud, cobwebs, roost-site substrate, ectoparasites, etc. If in doubt, e-mail close-up photos to your NWHC contact.

1. If the affected bat is a nonendangered or nonthreatened species, consider euthanasia for diagnostic evaluation at NWHC. Take close-up photos of affected individuals prior to handling. Collect up to 5 affected bats per location and contact NWHC prior to submission. Recommend guidelines for humane bat euthanasia are available at: www.michigan.gov/documents/emergingdiseases/**Humane_Euthanasia**_of_**Bats**-Final_244979_7.pdf

2. If the affected bat is an endangered or threatened species, photograph the affected individual prior to nonlethal sample collection and record information on data sheet (APPENDIX B). Next, collect a nonlethal sample for diagnostic evaluation. Options include a fungal tape lift from the most prolific fungal growth on the bat, preferably the muzzle (APPENDIX C) or punch biopsies from affected portions of the flight membranes only (APPENDIX D). Collect samples from up to3 individuals per site, place a wing band ID, and release. Contact NWHC prior to submission.

Hibernating bat species (flying adults) with evidence of wing damage (Wing Damage Index ≥ 2) caught opportunistically between June – July in states with unknown history of WNS

Protocols: Wing-Damage Index (http://www.fws.gov/WhiteNoseSyndrome/research.html)

1. Hibernating bat species (particularly *M. lucifugus, M. septentrionalis, P. subflavus*) with evidence of moderate to severe wing damage collected between June-July from states not previously known to have WNS may be submitted. Wing damage is defined as multifocal depigmentation or increased translucency of flight membranes involving more than 50% of the wing surface area. Tears or holes in the membranes may also be present. Whole carcasses (3-5 bats per site) or punch biopsies (2 per animal, maximum 5 bats) are acceptable.

2. If the affected bat is an endangered or threatened species, photograph the affected individual prior to nonlethal sample collection and record information on data sheet (APPENDIX B). Next, collect 1 punch biopsy from affected portion of each wing membrane only (APPENDIX D) for diagnostic evaluation. Collect samples from up to3 individuals per site, place a wing band ID, and release. Contact NWHC prior to submission.

3. Individual bats previously banded in WNS affected areas captured in unconfirmed states with current evidence of wing lesions may be submitted for evaluation. Whole, fresh carcass is ideal. If no lesions are present, punch biopsies of each wing membrane are advised. Information regarding band origination may be available by contacting the regional bat working groups.

   Southeast Bat Diversity Network (Eric Britzke 864-634-3641 or Susan Loeb 864-656-4865)
   Midwest Bat Working Group: John.Whitaker@indstate.edu
   Northeast Bat Working Group: list@list.wpunj.edu
   Western Bat Working Group: Rita Dixon 208-761-4958

Live bats from the groups listed below with evidence of wing damage (Wing Damage Index ≥ 2) opportunistically caught during other activities between June – October from WNS affected areas only (see map Appendix A)

Protocols: Wing-Damage Index (http://www.fws.gov/WhiteNoseSyndrome/research.html)

1. NWHC will accept whole carcasses (3-5 bats) or wing punch biopsies from nonhibernating bat species (Genera: *Lasiurus*, *Lasionycteris, Corynorhinus, Nycticeius, etc*) sampled in WNS positive states (NY, VT, MA, CT, PA, NJ, VA, WV, TN, MD, NH) during June – October and observed to have moderate to severe wing damage. Wing damage is defined as multifocal depigmentation or increased translucency of flight membranes involving more than 50% of the wing surface area. Tears or holes in the membranes may also be present.

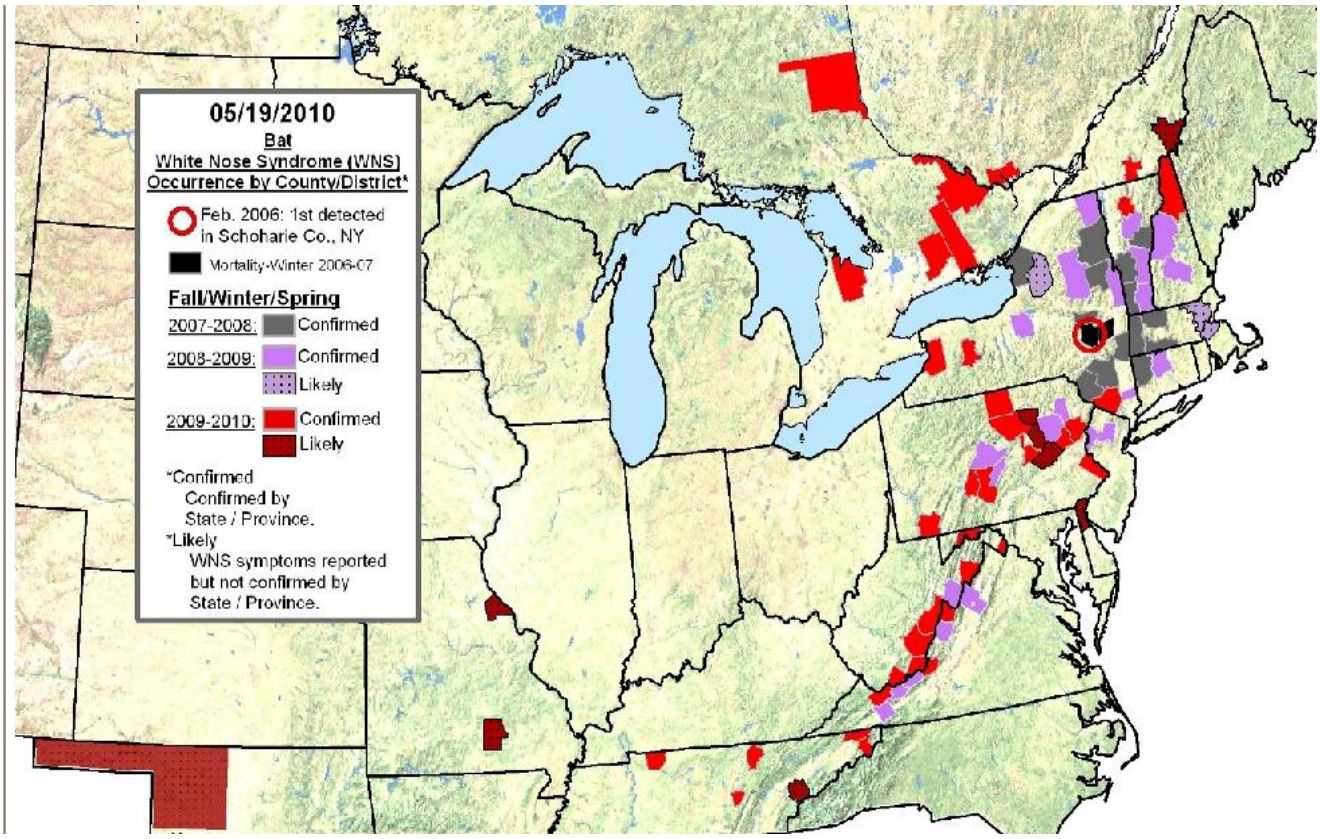
2. Only bat species not previously documented with WNS opportunistically captured on the landscape with moderate to severe wing damage (as described above) should be submitted for evaluation from previously confirmed WNS positive states.

DO NOT submit the following 6 species from WNS positive states if found outside caves/mines with wing damage:

- Little brown bats (*Myotis lucifugus*)
- Tricolored bats (*Perimyotis subflavus*)
- Northern long-eared bats (*Myotis septentrionalis*)
- Indiana bats (*Myotis sodalis*)
- Small-footed bats (*Myotis leibii*)
- Big brown bats (*Eptesicus fuscus*)

3. Male bats (preferably Myotis species) sampled from known WNS positive and/or *G. destructrans* positive hibernacula during the summer may be submitted for diagnostic evaluation for WNS. Submit 3 bats per site (limited to 2 sites per WNS affected state). Bats with suspicious wing lesions or multifocal UV fluorescence patterns should be targeted for sample submission. Whole carcasses are preferred to perform a variety of diagnostic tests.

APPENDIX A



Most current map updates are posted at http://www.fws.gov/WhiteNoseSyndrome/maps.html

Map by Cal Butchkoski, PA Game Commission

APPENDIX B – USGS NWHC Summer/Fall 2009 Bat Submission Datasheet

Date (MMDDYY):___________________
Location ID:_______________________
(*circle one*: maternity colony; bachelor colony; night roost; day roost; hibernaculum; other_______________________)
County:________________
State:_______
Decimal degrees (NAD83): N______________ E______________
Collector:_____________________ e-mail: ___________________

Estimated number of live bats at site___________; Estimated number of dead bats__________
Bats present (G. species) & estimated % of total popn: ________________ (____%);________________ (____%); ________________ (___%); ________________ (____%); __________________ (____%)
Percent of total population affected by clinical signs: ______________
Percentage of each species affected: ________________ (____%);________________ (____%); ________________ (____%);________________ (____%);________________ (____%)
Distribution pattern of affected bats at site: solitary *vs.* clustered;
(circle one from each row) outer periphery *vs.* inner region *vs.* throughout site

ID or Band# (state, MMDDYY, collector, ###)	**Species**	**Sex** *(circle one)*	**Status** (Live, Dead, Euth)	**Age Class** (Juv, Adult, Unknown)	**Weight (g)**	**Forearm length (mm)**	**Reichard Wing Score** *(circle one)*	**Photo file ID**	**Disposition** (Released, Fungal Tape, Wing Biopsy, Archived, Submitted)	**Comments/Notes**
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	
		M F	L D E	J A U			0 1 2 3		R T B A S	

Additional Notes/Diagrams:

APPENDIX C - Fungal tape-lift protocol for bats

Protocol: Tape-Strip Sampling of Bats for Identification of *Geomyces destructans* Fungal Infection

Authors: David S. Blehert and Anne Ballmann, USGS – National Wildlife Health Center

Date: 7 October 2009 (modified)

Purpose: The following procedure is designed to collect fungi from the skin of bats for later microscopic analyses while minimizing harm to the sampled bat.

Required materials:

NOTE- Neither the USGS nor the NWHC endorse these vendors as the only sources of these products. This information is provided only as a guideline.

1) Glass microscope slides with white label (25 mm (W) X 75 mm (L); 1 mm thick). Fisher Scientific Catalog #12-552. Fisher list price $58.34 pack (144/pack).

2) Fungi-Tape (25 yards X 1 inch; approximately 1 mm thick). Fisher Scientific Catalog #23-769-321 (Scientific Device Laboratory No. 745). Fisher list price $35.59 per box.

3) Plastic 5-slide transport mailers. (Maximum capacity is 10 slides per mailer – see instruction #9 below). Fisher Scientific Catalog #12-569-35 ($31.00 for pack of 25) or #12-587-17B ($185.35 for pack of 200).

4) Pencil

Procedure:

1) Wear new disposable gloves when handling each individual bat to reduce the risk of cross-contamination.

2) Label the end of a microscope slide in pencil with an animal ID number, date, and anatomical sample location.

3) Remove a precut piece of Fungi-Tape from the box being careful not to contaminate the adhesive surface.

4) Bend the tape-strip (without creasing), adhesive-side out, between your thumb and index finger so that the tape forms the shape of a "U" (Fig. 1).

5) Sample muzzles of bats with grossly visible blooms of fungal growth. When possible, avoid collecting samples from wing membranes as analyses of unfurred skin have not been reliable in detection of *Geomyces destructans*.

6) Lightly touch the adhesive surface of the tape-strip, at the bottom of the "U", to an area of suspect fungal growth on bat surface (Fig. 2). DO NOT use your finger to press the tape down onto the bat's muzzle. Attempt to maximize adherence of fungus to the tape adhesive while minimizing adherence of hair (Fig. 3).

7) If only a small area is transferred to the tape, use a different portion of the same tape "U" to touch another area of visible fungal growth on the bat. DO NOT attempt to obtain more than 3 lifts per tape strip. **Collect only 1 tape-strip per live bat.**

8) Align the tape-strip containing the fungal sample, adhesive-side down, over the microscope slide. Ensure that the edges of the tape-strip do not protrude beyond the edges of the microscope slide when laid flat, and do not remove any portion of the tape-strip from the glass slide once it has adhered (Fig. 4).

9) Lightly wipe over the top surface of the tape-strip using a clean paper or cloth towel to consistently adhere the strip to the slide. Circle the area of tape used to transfer the fungus with a permanent marker.

10) Place each slide into a slide mailer for safe transport. If 2 slides are placed per slot, ensure that the tape surfaces of each slide are facing outwards (only the non-tape sides should be in contact so as not to crush the tape). Seal the slide mailer shut with standard tape or rubber bands prior to shipment.

11) Place slide mailer(s) into a clean Ziploc bag and seal closed to transport from the hibernaculum. Place in a second Ziploc bag

12) The slide mailers can now be held at ambient temperature and shipped to the NWHC for microscopic examination. Ship mailers in a padded envelop with a completed specimen history form. If including slide mailers in a cooler shipment with bat carcasses, ensure that the slide mailers are not in contact with the blue ice. Send an electronic copy of the completed specimen history form to LeAnn White (clwhite@usgs.gov) (or Anne Ballmann (aballmann@usgs.gov) after December 14, 2009). Contact Anne (608-270-2445) or LeAnn (608-270-2491) if you have any additional questions.

APPENDIX C. Illustrations – Fungal tape-lift protocol for bats

-Photographs by D. Berndt and D. Johnson, USGS – NWHC

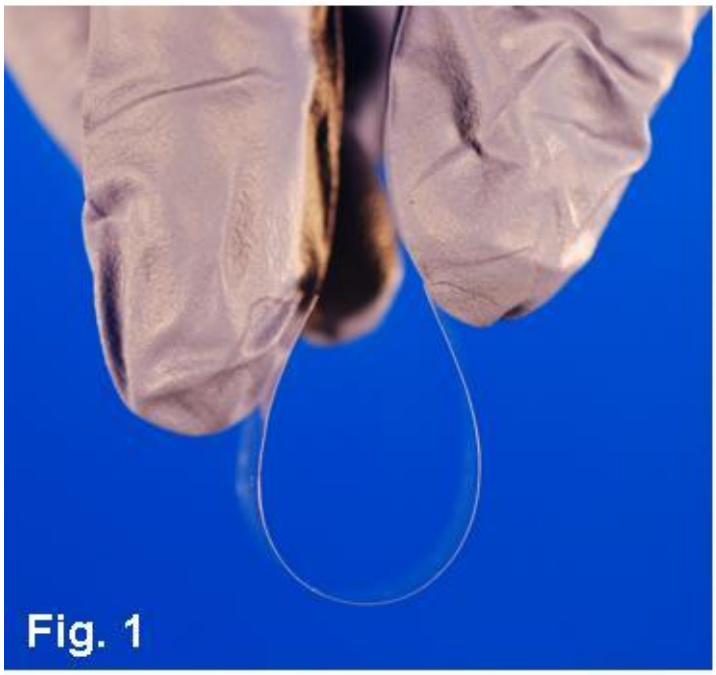


Fig. 1

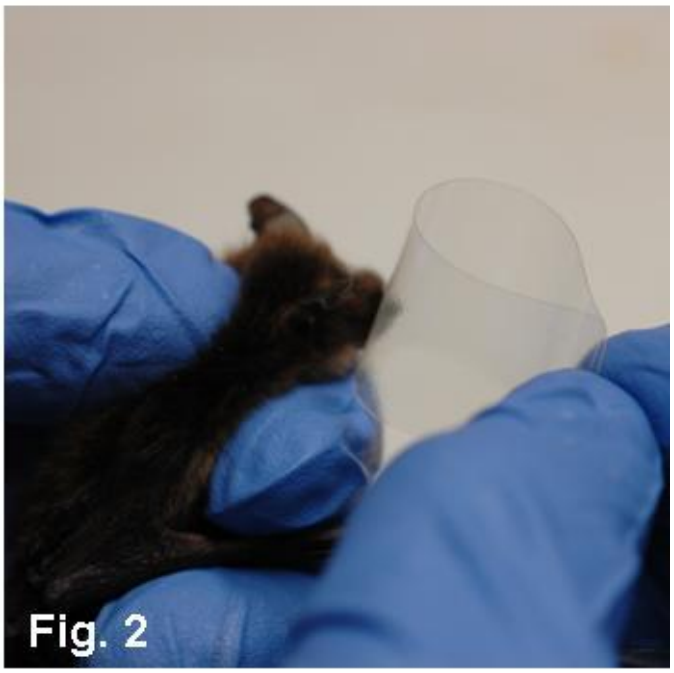


Fig. 2

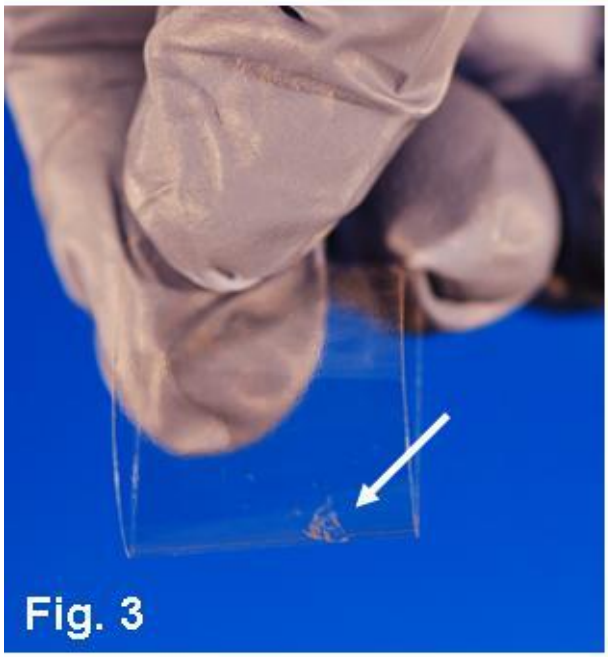


Fig. 3

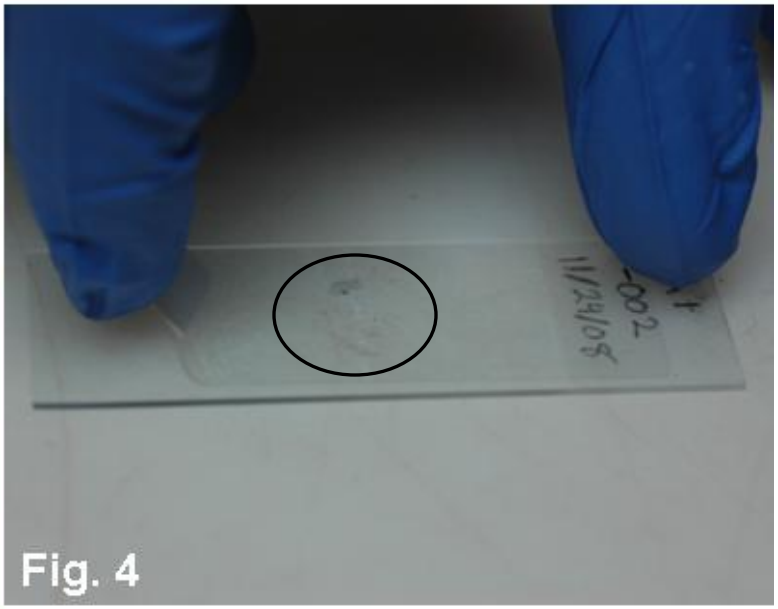


Fig. 4

Appendix D - Instructions for Taking a Tissue Biopsy

Updated by Pat Ormsbee and Jan Zinck 5/14/09 (original: Shonene Scott, Portland State University 5/2003)
Modified by Anne Ballmann 6/10/10

NOTE: If punch biopsies are the only sample type to be submitted to the lab for PCR testing of G. destructans in a particular case, it is highly recommended that 2 biopsies per bat be collected (from different wings). Additional population genetic sampling should not be attempted in these individuals to reduce the number of holes in the wings.

1. When taking biopsies it is important to reduce the potential for cross-contamination between bats. In order to do this, use a small clean piece of sturdy cardboard that can be discarded after each animal, a new tissue punch for each sample, sterilized forceps, and disposable gloves.

2. Label a sterile vial: Use a black ultra-fine Sharpie permanent marker and a sticky paper label. Be careful that once the label is adhered to the tube the entire identifier is visible. Use the following naming convention to uniquely identify the bat:

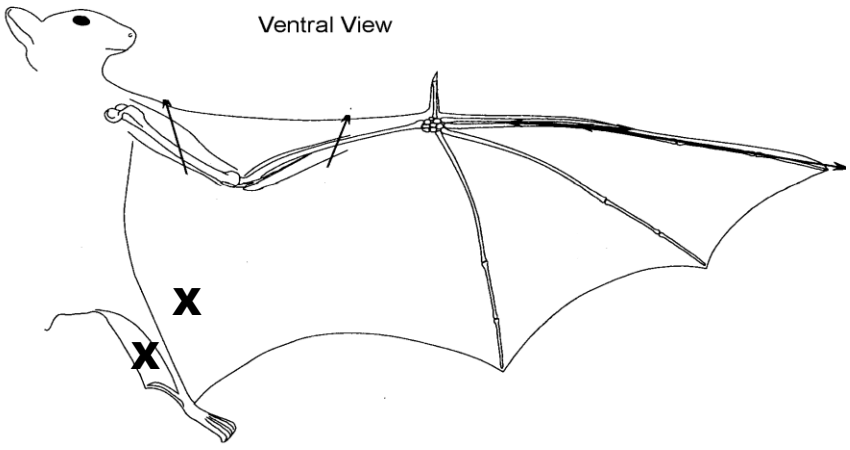
3. State, Date (MMDDYY), Collector initials, bat number (ex: WI061609AEB001)

4. Have a fresh cardboard square, a labeled tube, a new tissue punch, and a sterilized forceps ready. Do not touch (contaminate) the end of the punch, the forceps, or the inside of the tube lid with fingers or environmental debris.

5. Identify 2 representative lesions to biopsy on the affected wings/tail of the bat. Place the bat on the cardboard on its back and extend one wing membrane (Avoid sampling from bats with large wing tears). For people inexperienced in this technique, it works best when one person holds the bat and another person collects the biopsy.

6. When collecting wing tissue biopsies, avoid bones and major blood vessels. (Figure 1). <u>If possible, locate an affected area near the body wall</u> within the lower half of the wing membrane or uropatagium. Press the punch firmly through the membrane and twist the punch slightly to ensure a complete punch. Apply direct pressure to biopsy site for several minutes if bleeding occurs.

Figure 1: "X" marks ideal sample locations for collecting tissue biopsies from a bat flight membranes.



6. Carefully lift the bat off the biopsy board and look for the tissue sample. It should either be on the board or inside the tip of the punch. Be careful on windy days since the wind can blow the tissue off of the board. A new 25 ga needle or sterile forceps can be used to pick up the tissue and transfer each biopsy to separate storage vials which contain no storage media.

7. Release the bat only after tissue samples have been placed into the tubes, the tubes have been closed, and any bleeding has stopped. The number of biopsies has been limited to 2 per bat to prevent compromising flight.

8. While in the field, sample tubes should be stored on ice. Subsequently, samples should be frozen until submitted for fungal PCR analysis.

9. Dispose of the used biopsy punch after each animal. DO NOT reuse the same biopsy punch on multiple bats. The punches are very sharp. Be careful to not cut yourself. Change into new gloves before handling each bat.

10. Before reusing forceps while in the field, follow the flame sterilization protocols described in "Disinfection Protocol for Bat Field Research/Monitoring, June 2009" (http://www.fws.gov/northeast/wnsresearchmonitoring.html). Upon returning to the office, perform a more thorough cleaning and disinfection of nondisposable biopsy equipment with detergent washing followed by soaking in a 10% bleach solution for 10 min with a thorough clean water rinse. Once dry, forceps can be placed into a clean hard surface container (not plastic bags), free of contaminates, marked for cleaned forceps, and with handles all pointing in the same direction.

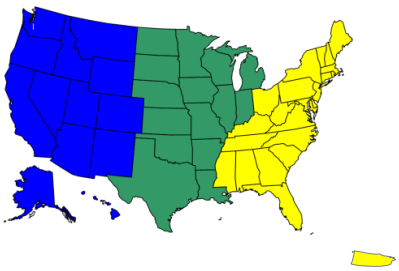
11. Ship wing tissues to NWHC: ensure that all cryovials are labeled and lids are secured in place to prevent cross-contamination of samples. Wrap lid of cryovials in parafilm and place in a Ziploc bag. If parafilm is not available double-bag specimens before placing in cooler. Specimens should be chilled and shipped overnight in a cooler with blue ice. If samples cannot be shipped overnight freeze them and ship as soon as possible.
Send an electronic copy of the completed specimen history form or datasheet to the appropriate NWHC contact . Specimen history form, shipping address, and examples of appropriate shipping materials are in Appendix E. Contact Anne Ballmann (aballmann@usgs.gov , 608-270-2445) if you have any additional questions.

SUPPLIES: NOTE- Neither the USGS nor the NWHC endorse these vendors as the only sources of these products. This information is provided only as a guideline

- 2 mm biopsy punches Fisher Scientific Catalog # NC9515874 ($106.73/pack of 50)
- Forceps **OR** 25 gauge needles and sharps collection container
- 10% bleach solution (can be made fresh each time, or can be stored in opaque containers for 24 hours, it begins to break down after this)
- Sterile rinse water
- 5 ml sterile plastic vials with caps
- 95% ethanol and flame source such as cigarette lighter (for sterilizing metal sampling equipment)
- Fine point permanent marker
- Vial labels
- Disposable gloves
- Paper towels/gauze
- Nonporous cutting board
- Ziploc bags and cooler with blue ice.

Appendix E

National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711
Phone: 608.270.2400
FAX: 608.270.2415



SPECIMEN HISTORY FORM

For mortality events please e-mail a USGS Field Investigation Team member before shipping
Western States: Krysten Schuler kschuler@usgs.gov, 608-270-2447
Central States: LeAnn White clwhite@usgs.gov, 608-270-2491
Eastern States: Anne Ballmann aballmann@usgs.gov , 608-270-2445
For single animal cases, Nationwide: Jennifer Bradsby jbradsby@usgs.gov, 608-270-2443

Submitter's name: **Telephone:**
Address:

E-mail:

Collector's Name: **Affiliation:**

Telephone:

E-mail:

Date collected:
Method of animal collection: ☐ **Found Dead,** ☐ **Died in Hand,** ☐ **Euthanized**
Method of euthanization:
Species:
Number Submitted: **Condition:** ☐ **Chilled,** ☐ **Frozen,** ☐ **Preserved Tissues**

Specific die-off location (refuge unit, pond, address, intersection, park, etc):

State: **County:** **Nearest City:**

Latitude/longitude (Decimal degree in WGS 84): **Zone:**

Disease onset date: (Best estimate) **Disease end date:** (best estimate)

Species affected: (The diversity of species affected may provide clues to the disease involved.)

Age/sex: (Any pattern noticed that is related to age and sex?)

Known dead: (Actual number counted) **Known sick:**

Estimated dead: **Estimated sick:**
(Consider removal by scavengers or other means, density of vegetation, etc.)

Clinical signs: (Any unusual behavior and physical appearance.)

Population at risk: (Number of animals in the area that could be exposed to the disease.)

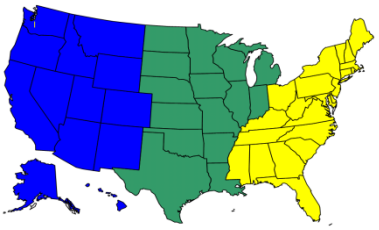
Population movement: (Recent changes in number of animals on area and their source or destination, if known.)

Problem area description: (Land use, habitat types, and other distinctive features.)

Environmental factors: (Record conditions such as storms, precipitation, temperature changes, or other changes that may contribute to stress.)
Comments: (Additional information/observations of value such as past occurrences of disease in area, photographs or videos)

Appendix F **USGS – National Wildlife Health Center**

INSTRUCTIONS FOR COLLECTION AND SHIPMENT OF AVIAN AND MAMMALIAN CARCASSES



Contact your USGS Field Investigation Team (FIT) member first!
Eastern states – Dr. Anne Ballmann aballmann@usgs.gov 608-270-2445
Central states - Dr. LeAnn White clwhite@usgs.gov 608-270-2491
Western states – Dr. Krysten Schuler kschuler@usgs.gov 608-270-2447
Single animal cases, Nationwide: Jennifer Bradsby jbradsby@usgs.gov,
Emergency Contact Number 608-270-2400

The following instructions should be used for collecting and shipping wildlife carcasses, carcass parts, and samples extracted from animals to the National Wildlife Health Center (NWHC) to insure adequate and well preserved specimens. Freezing/thawing impedes isolation of some pathogens and damages tissues. NWHC prefers unfrozen specimens if they can be sent within 24-36 hours of collection or death. We will provide guidance on freezing samples on a case-by-case basis. As a general guideline: if you cannot call or ship within 24-36 hours, freeze the animal(s).

☐ Contact FIT to get shipping approval and discuss shipping arrangements. Typically, ship specimens by 1-day (overnight) service, Monday through Wednesday, to guarantee arrival at NWHC before the weekend. If specimens are fresh and need to be shipped on Thursday or Friday, special arrangements can be made.

☐ Email/fax history and tracking number to FIT. Packages will not be opened if history does not arrive first!

☐ Use rubber, vinyl, or nitrile gloves when picking up sick or dead animals. If you do not have gloves, insert your hand into a plastic bag.

☐ More than one disease may be affecting the population simultaneously. When possible, collect both sick and dead animals. Note behavior of sick animals before euthanizing.

☐ Collect specimens that are representative of all species affected and geographic areas.

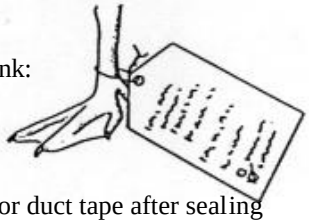
☐ Collect the freshest dead specimens. Decomposed or scavenged carcasses are usually of limited diagnostic value. If you plan to collect animals in the field, take along a cooler containing ice to immediately chill carcasses.

☐ Contact NWHC for assistance when collecting samples from animals that are too large to ship.

☐ Collect animals under the assumption that an infectious disease or toxin is involved and other animals may be at risk. Protect yourself as some diseases and toxins are hazardous to humans.

☐ Immediately attach a leg tag to each animal with the following information in pencil or waterproof ink:

- Date collected
- Species
- Location (specific site, town, county, state)
- Found dead or euthanized
- Collector (name/address/phone)
- Your reference #



☐ Place each animal in a plastic bag, close, and seal the bag. Cover zipper bag closure with strapping or duct tape after sealing zipper.

Twist non-zipper bags closed, fold over on itself, and secure with package strapping or duct tape.

☐ Place 1st bag inside a 2nd bag, close and seal. More than one individually bagged animal can be placed in the 2nd bag. This prevents cross-contamination of individual specimens and leaking shipping containers.

☐ Tag the outside of 2nd bag and number of animals and type, date collected, location, and name of collector. Reminder order: TAG, BAG, BAG, TAG.

- ☐ Use a hard-sided cooler in good condition for shipment. Close the drain plug of cooler and tape over inside. Line cooler with a thick bag (1 mil thickness, 3rd layer of bags).
- ☐ Place absorbent material in the 3rd plastic bag to absorb any liquids that might leak during shipping.
  See appendix for examples of bags and absorbent materials.
- ☐ Pack the individually bagged animal(s) that are contained within the 2nd sealed bag into the 3rd bag with enough FROZEN BLUE ICE PACKS or similar coolant to keep carcasses cold. Use enough coolant to keep samples chilled if there is a delay in delivery.
  - Blue ice (unfrozen) can be obtained at hardware, sporting goods, or grocery stores.
  - Wet ice can be used if frozen in a sealed plastic container (i.e., soda or water bottle).
  - DO NOT USE DRY ICE.
- ☐ Seal the 3rd bag with methods described for 1st bag.
- ☐ Place the completed specimen history and return shipping label in a ziplock bag and tape to the inside lid of the cooler (if you want the cooler returned). NWHC CANNOT PAY FOR SHIPPING.
- ☐ Using packing or duct tape, tape the cooler shut around the lid and at each end using a continuous wrap around the cooler.
- ☐ Attach the shipping document (airbill) with the DOT information below to the outside of each cooler in a resealable pouch:

  Address:

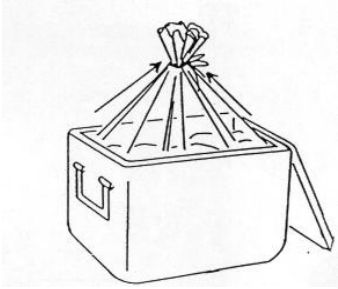
  National Wildlife Health Center
  Necropsy Loading Dock
  6006 Schroeder Road
  Madison, WI 53711

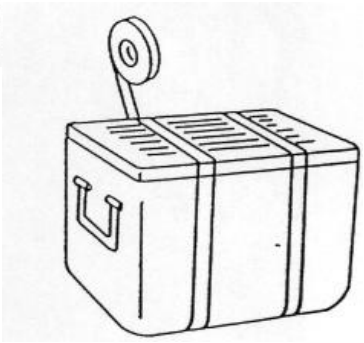
  Emergency Contact:

  NWHC FIT emergency
  608-270-2400

  Supplementary Labels**:**

  Keep Cold





- ☐ Mark the cooler with the appropriate information:

  (See Pg. 3 for printable marking labels)

  - Carcasses of animals that died of unknown causes:

    BIOLOGICAL SUBSTANCE, CATEGORY B and **UN 3373.**
  - Blood and tissue samples from apparently healthy animals (hunter-killed, live captured):

    EXEMPT ANIMAL SPECIMENS.
  - Blood and tissue samples from dead or sick animals:

    BIOLOGICAL SUBSTANCE, CATEGORY B and **UN 3373**.

. Note the tracking number in case packages are delayed.

- ☐ These instructions cover federal shipping regulations for commercial carriers.

Appendix:

Example of bags available at large supermarkets (list not all inclusive):

Inner and second layer bags:

Hefty Big Bag – 22 gal	Ziplock Freezer – 1 gallon
Hefty Freezer – 1 gal	Ziplock Big Bag – 20 gallon
Hefty Jumbo – 2.5 gal	Glad Freezer – 1 qt, 2 qt, 1 gal

Third layer for cooler liner:

Hefty Cinch Sak (1.1 mil) – 33 and 39 gal	Glad Force Flex (1.05 mil) – 25 gal
Hefty Lawn and Leaf (1.1 mil) – 33 and 39 gal	Hefty Ultra Flex (1.3 mil) – 30 gal
House brand large trash (1.1 mil) – 30 gal	House Lawn - Leaf (1.2 mil) – 39 gal

Absorbent material:

Super absorbent packet or pads for water	Cellulose wadding
Paper towels	Cotton batting or cotton balls

Do not use packing peanuts or shredded paper.

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BIOLOGICAL SUBSTANCES, CATEGORY B

EXEMPT ANIMAL SPECIMENS